

Listing of pending claims renumbered for Examiner's Amendment

1. (Previously presented) A recombinant replicon nucleic acid comprising:
 - a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
 - b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
 - c)
an alphavirus subgenomic promoter-IRES-heterologous nucleic acid of interest (NOI)
cassette; and
 - d) a nucleic acid encoding a 3' alphavirus replication recognition sequence.
2. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2, nsp3 and nsp4.
3. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2 and nsp3 and wherein the recombinant replicon nucleic acid comprises nucleotide sequence encoding alphavirus nonstructural protein nsp4 that is not contiguous with the nucleic acid sequence of (b).
4. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the IRES is selected from the group consisting of cellular IRESs, plant IRESs, mammalian virus IRESs, synthetic IRESs and insect virus IRESs.
5. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the alphavirus subgenomic promoter of (c) is a minimal or modified alphavirus subgenomic promoter.
6. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the heterologous NOI of (b) encodes a protein or peptide.

7. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the heterologous NOI is an antisense sequence.
8. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the heterologous NOI encodes a ribozyme.
9. (Previously presented) The recombinant replicon nucleic acid of claim 1, further comprising a nucleotide sequence encoding an alphavirus structural protein.
10. (Previously presented) The recombinant replicon nucleic acid of claim 9, wherein the alphavirus structural protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.
11. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid sequence of (a) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.
12. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid sequence of (b) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.
13. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the alphavirus subgenomic promoter of (c) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.
14. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid sequence of (d) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

15. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c), such that at least 80% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

16. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c) such that at least 10% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

17. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid is RNA.

18. (Previously presented) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid is DNA.

19. (Previously presented) A recombinant replicon nucleic acid comprising:
a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
c) an alphavirus subgenomic promoter-IRES-heterologous nucleic acid of interest (NOI) cassette, said cassette further comprising a spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES; and
d) a nucleic acid encoding a 3' alphavirus replication recognition sequence.

20. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is at least 30 nucleotides in length.

21. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is between 25 and 7500 nucleotides in length.

22. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is between 150 and 1000 nucleotides in length.

~~23~~25. (Currently amended) A population of infectious, defective, alphavirus particles, wherein each particle comprises the alphavirus particle of claim ~~27~~23, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

~~24~~26. (Currently amended) A population of infectious, defective, alphavirus particles, wherein each particle comprises the alphavirus particle of claim ~~28~~24, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

~~25~~27. (Currently amended) A pharmaceutical composition comprising the population of claim ~~23~~25 in a pharmaceutically acceptable carrier.

~~26~~28. (Currently amended) A pharmaceutical composition comprising the population of claim ~~24~~26 in a pharmaceutically acceptable carrier.

~~27~~23. (Currently amended) An alphavirus particle comprising the recombinant replicon nucleic acid of claim 1.

~~28~~24. (Currently amended) An alphavirus particle comprising the recombinant replicon nucleic acid of claim 19.

29. (Currently amended) The alphavirus particle of claim ~~27~~23, comprising an attenuating mutation.

30. (Currently amended) The alphavirus particle of claim ~~28~~24, comprising an attenuating mutation.
31. (Previously presented) The recombinant replicon nucleic acid of claim 1, comprising an attenuating mutation.
32. (Previously presented) The recombinant replicon nucleic acid of claim 19, comprising an attenuating mutation.
33. (Currently amended) A population of infectious, defective, alphavirus particles, comprising the alphavirus particle of claim ~~27~~23.
34. (Currently amended) A population of infectious, defective, alphavirus particles, comprising the alphavirus particle of claim ~~28~~24.
35. (Previously presented) A composition comprising the population of claim 33, in a pharmaceutically acceptable carrier.
36. (Previously presented) A composition comprising the population of claim 34, in a pharmaceutically acceptable carrier.
37. (Previously presented) A method of making infectious, defective alphavirus particles, comprising:
- a) introducing into a cell the following:
 - (i) the recombinant replicon nucleic acid of claim 1, and
 - (ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the one or more helper nucleic acids produce all of the alphavirus structural proteins; and
 - b) producing the alphavirus particles in the cell.

38. (Previously presented) The method of claim 37, wherein the recombinant replicon nucleic acid further comprises a nucleotide sequence encoding an alphavirus structural protein.

39. (Previously presented) The method of claim 37, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

40. (Previously presented) The method of claim 37, wherein the helper nucleic acid is a recombinant nucleic acid comprising a promoter and nucleotide sequences encoding one or more alphavirus structural proteins.

41. (Previously presented) The method of claim 40, wherein the helper nucleic acid is DNA.

42. (Previously presented) The method of claim 41, wherein the promoter is a CMV promoter.

43. (Previously presented) The method of claim 41, wherein the helper nucleic acid comprises nucleotide sequences encoding all of the alphavirus structural proteins.

44. (Previously presented) The method of claim 37, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an IRES element, a nucleotide sequence encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

45. (Canceled).

4648. (Currently amended) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, an alphavirus subgenomic promoter, a heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise the recombinant nucleic acid of claim 4846, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

47. (Previously presented) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) the recombinant replicon RNA of claim 1; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise a recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes one or more alphavirus structural proteins;

c) and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

4846. (Currently amended) A recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes one or more alphavirus structural proteins; and

c) a 3' alphavirus replication recognition sequence.

49. (Currently amended) A cell comprising the recombinant nucleic acid of claim ~~48~~46.

50. (Previously presented) The recombinant replicon nucleic acid of claim 1, further comprising an alphavirus packaging signal.

51. (Previously presented) The recombinant replicon nucleic acid of claim 19, further comprising an alphavirus packaging signal.

52. (Previously presented) A recombinant nucleic acid comprising: a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES, wherein the NOI encodes one or more alphavirus structural proteins;

c) and a 3' alphavirus replication recognition sequence.

53. (Currently amended) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim ~~23~~25.

54. (Currently amended) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim ~~24~~26.

55. (Currently amended) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim ~~25~~27.

56. (Currently amended) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the composition of claim ~~26~~28.

57. (Canceled).

58. (Previously presented) A recombinant replicon nucleic acid comprising:
a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
c) a first alphavirus subgenomic promoter-IRES-heterologous NOI cassette;
d) a second alphavirus subgenomic promoter-IRES-heterologous NOI cassette; and
e) a nucleic acid encoding a 3' alphavirus replication recognition sequence.

59. (Previously presented) The recombinant replicon nucleic acid of claim 58, further comprising an alphavirus packaging signal.

60. (Canceled).

61. (Previously presented) A recombinant replicon nucleic acid comprising:
a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
c) a first alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a first spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES;
d) a second alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a first spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES; and
e) a nucleic acid encoding a 3' alphavirus replication recognition sequence.

62. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2, nsp3 and nsp4.

63. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2 and nsp3 and wherein the recombinant replicon nucleic acid comprises a nucleotide sequence encoding alphavirus nonstructural protein nsp4 that is not contiguous with the nucleic acid sequence of (b).

64. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the IRES is selected from the group consisting of cellular IRESs, plant IRESs, mammalian virus IRESs, synthetic IRESs and insect virus IRESs.

65. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the alphavirus subgenomic promoter of (c) is a minimal or modified alphavirus subgenomic promoter.

66. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI of (b) encodes a protein or peptide.

67. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI is an antisense sequence.

68. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI encodes a ribozyme.

69. (Previously presented) The recombinant replicon nucleic acid of claim 19, further comprising a nucleotide sequence encoding an alphavirus structural protein.

70. (Previously presented) The recombinant replicon nucleic acid of claim 69, wherein the alphavirus structural protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

71. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (a) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

72. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (b) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

73. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the alphavirus subgenomic promoter of (c) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

74. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (d) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

75. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c), such that at least 80% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

76. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c) such that at least 10% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

77. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid is RNA.

78. (Previously presented) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid is DNA.

79. (Previously presented) A cell comprising the recombinant replicon nucleic acid of claim 19.

80. (Previously presented) A cell comprising the recombinant replicon nucleic acid of claim 1.

81. (Previously presented) A cell comprising the recombinant nucleic acid of claim 52.

82. (Previously presented) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

- (i) the recombinant replicon nucleic acid of claim 19, and
- (ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the one or more helper nucleic acids produce all of the alphavirus structural proteins; and

b) producing the alphavirus particles in the cell.

83. (Previously presented) The method of claim 82, wherein the recombinant replicon nucleic acid further comprises a nucleotide sequence encoding an alphavirus structural protein.

84. (Previously presented) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

85. (Previously presented) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a promoter and nucleotide sequences encoding one or more alphavirus structural proteins.

86. (Previously presented) The method of claim 85, wherein the helper nucleic acid is DNA.

87. (Previously presented) The method of claim 85, wherein the promoter is a CMV promoter.

88. (Previously presented) The method of claim 85, wherein the helper nucleic acid comprises nucleotide sequences encoding all of the alphavirus structural proteins.

89. (Previously presented) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an IRES element, a nucleotide sequence encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

90. (Previously presented) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, an alphavirus subgenomic promoter, a heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise the recombinant nucleic acid of claim 52, whereby all of the alphavirus structural proteins are produced in the cell; and
b) producing the alphavirus particles in the cell.

91. (Previously presented) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) the recombinant replicon RNA of claim 19; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise a recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes- one or more alphavirus structural proteins;

c) and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

92. (Previously presented) The recombinant nucleic acid of claim 6, wherein the peptide is an immunogen.

93. (Previously presented) The recombinant nucleic acid of claim 66, wherein the peptide is an immunogen.